Asian Journal of Chemistry Vol. 20, No. 4 (2008), 2515-2520

Synthesis and Biological Evaluation of Schiff Bases of 2-Amino-5-(2-chlorophenyl)-1,3,4-oxadiazole

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Few Schiff bases of 2-amino-5-(2-chlorophenyl)-1,3,4-oxadiazoles have been synthesized using aromatic aldehydes. The chemical structures of these compounds were confirmed by elemental analysis, IR and ^{13}C NMR. These compounds were tested against representatives of Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*), yeast (*Candida albicans*) and mould (*Aspergillus fumigatus*). Ciprofloxacin and clotrimazole were used as standard drug for anti-microbiological and antifungal activities, respectively.

Key Words: Synthesis, Microbial activities, Schiff bases, 2-Amino-5-(2-chlorophenyl)-1,3,4-oxadiazole.

INTRODUCTION

1,3,4-Oxadiazoles have been reported to posses a variety of activities like antianxiety[1], antidepressant[2], antiinflammatory[3], antimicrobial and antifungal[4-8] activities. As a continuation of our research program on biologically active heterocyclic, we synthesized new compounds bearing an azomethine linkage and evaluated *in vitro* their antimicrobial and antifungal activities. This paper reports the evaluation of new Schiff bases (azomethine) bearing furan and substituted phenyl substituents.

EXPERIMENTAL

The melting points of the compounds were determined with a Stuart-SMP3 apparatus and are uncorrected. ^{13}C NMR spectra were recorded on Bruker DPX 300 instrument (75 MHz) using TMS as internal standard (Chemical shifts are given in ppm). IR spectra were recorded in KBr discs on an Ati-Matson Genesis FT-IR. C, H and N analyses were carried out on a Carlo Erba 1106 and Perkin-Elmer analyzer. The progress of the reaction was monitored by thin layer chromatography, with F254 silica gel precoated

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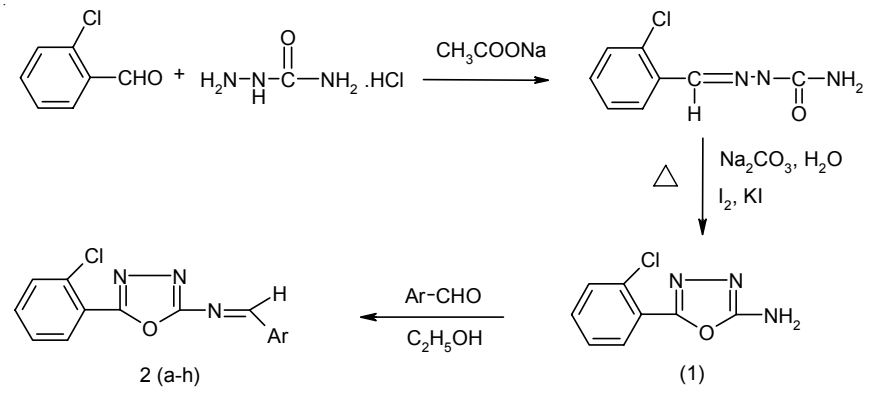
sheets (Merck) using chloroform:methanol (7:1) as eluent; UV light was used for detection.

General method for synthesis of Schiff bases: 2-Amino-5-(2-chlorophenyl)-1,3,4-oxadiazole (**1**) (m.p. 165-166 °C, lit.[9] 160-162 °C) were prepared by reported procedure for 1,3,4-oxadiazoles[9,10] (**Scheme-I**). The Schiff bases (**2**) were prepared by reacting (**1**, 0.01 M) with the selected aldehyde (0.011 M) under reflux for 6 h in absolute ethanol (40 mL). Nucleophilic addition of the -NH_2 group to the carbonyl function of the aromatic aldehyde is not straightforward. The poor reactivity of the starting materials was overcome with azeotropical removal of the water by a Dean-Stark trap. The alcohol was removed by distillation under vacuum and residue crystallized using absolute ethanol or benzene.



2 (a-h) (1)

Ar = *o*-chloropheny; *p*-chlorophenyl; *p*-methoxyphenyl; *o*-nitrophenyl; *p*-nitrophenyl; 5-nitrofuranyl; 5-phenyl-2-furanyl and 5-(2-nitro-phenyl)-2-furanyl

Scheme-I

Approximate lethal dose (ALD_{50}): The approximate lethal dose of the synthesized compounds were determined by using the Horn method[11].

Antibacterial activity: The chemical compounds were tested for antibacterial activity against Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*). The minimal inhibitory concentrations (MICs) of the chemical compounds were determined by the conventional broth dilution method using the two serial dilution technique[12]. Ciprofloxacin was used as standard antibacterial agent. Serial diluted solutions of the test compounds (200-0.39 µg/mL) and ciprofloxacin were prepared in DMSO. MIC tests were carried out in Muller-Hinton Broth (Difco) medium, pH 7.2, with an inoculum of (1-2) × 10^6 colony forming unit per mL (CFU/mL) for each microorganisms. The chemical compounds Muller-Hinton Broth serial tube dilutions inoculated with each

bacterium were incubated on a rotary shaker at 37 ± 1 ºC for 18 h at 100 rpm. Growth inhibition with concentration at 200 µg/mL or the lower were carried out in triplicate. All test tubes showing the positive or negative growth were confirmed by the agar plate method. The minimum inhibitory concentrations (MICs) were calculated as the average concentration of the test agent in the broth tube showing consecutive positive and negative growth. Broth medium containing DMSO inoculated microorganisms were used as a control.

Antifungal activity: Assays were carried out in Sabourdaud Dextrose Broth for *Candida albicans* and *Aspergillus fumigatus* with an inoculum of 5×10^5 colony forming unit mL (CFU/mL). The minimum inhibitory concentrations of the chemical compounds were determined after incubation at 28 ± 1 ºC for 36 h in the presence of serial dilution of test compounds. The minimum inhibitory concentrations of the chemical compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no growth (*i.e.*, no turbidity).

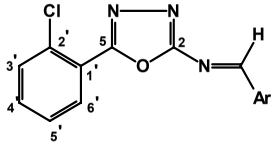
RESULTS AND DISCUSSION

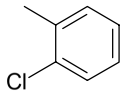
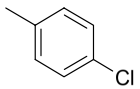
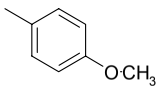
The physico-chemical data of the compounds are mentioned in Table-1. The infrared spectra of the final compounds **2** showed the characteristic absorption bands for C-O (1100 cm^{-1}), C=N group of the oxadiazole ring (*ca.* 1600 cm^{-1}) and imine (*ca.* 1530 cm^{-1}). IR spectra also confirm the presence of the functional groups in the final compounds. The structure of the final compounds **2** was supported by the ^{13}C NMR spectra. ^{13}C NMR of the final compounds exhibited peaks at about 173 and 168 ppm due to the carbon atom of oxadiazole ring. The peaks at *ca.* 110, 116, 141, 153 ppm are due to the presence of the carbon atoms of the furan ring. All other signals were in accordance to their expected chemical shift.

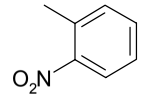
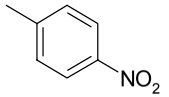
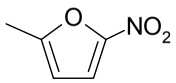
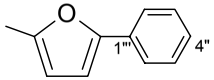
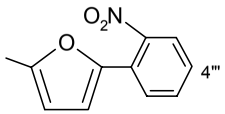
The ALD_{50} of the synthesized compounds were ranged from 464-825 mg/kg intraperitoneally (Table-2).

in vitro Antibacterial and antifungal screening results of synthesized compounds **2b-h** showed that compound **2e** is the most active derivative with MIC 88 µg/mL against *S. aureus*, 70 µg/mL (*E. coli*), 85 µg/mL (*P. aeruginosa*), 89 µg/mL (*C. albicans*) and 78 µg/mL (*A. fumigatus*). Other active compounds were **2b** (97 µg/mL against *S. aureus*, 100 µg/mL against *E. coli*, 102 µg/mL against *P. aeruginosa*, 95 µg/mL against *C. albicans* and 100 µg/mL against *A. fumigatus* Table-2) and **2a** (102 µg/mL against *S. aureus*, 108 µg/mL against *E. coli*, 112 µg/mL against *P. aeruginosa*, 102 µg/mL against *C. albicans* and 120 µg/mL against *A. fumigatus*). The compounds bearing furan ring (5-substitued furan), which were expected to have antibacterial and antifungal activity were less active than the chloro- or nitrophenyl derivatives (Table-2). Data represent the mean values for **3** independent determinations. Variation among the sample was less than 15%.

TABLE-1
PHYSICO-CHEMICAL DATA OF OXADIAZOLE SCHIFF BASES (**2**)



Compd.	Ar	m.f.	m.p. (°C)	Yield (%)	IR ν(cm^{-1})			^{13}C NMR δ (ppm), (DMSO-d_6, TMS)
					N=C	Azomethine C–H	Substituent	
2a		$C_{15}H_9N_3OCl_2$	178-180	72	1621.1, 1545.8	2914.2, 2825.6, 1378.5	–	136.7 (C-1'), 132.5 (C-2'), 129.3 (C-3'), 129.9 (C-4'), 127.0 (C-5'), 128.5 (C-6'), 169.1(C-5), 174.0(C-2), 163.5 (N=C), 131.5 (C-1"), 134.2 (C-2"), 128.9 (C-3"), 132.2(C-4"), 126.6 (C-5"), 130.3(C-6"),
2b		$C_{15}H_9N_3OCl_2$	199-200	73	1616.3 (ring), 1541.5 (C=N)	2919.7, 2826.5, 1382.6	–	136.8 (C-1'), 132.5 (C-2'), 129.5 (C-3'), 130.0 (C-4'), 127.0 (C-5'), 128.5 (C-6'), 169.2(C-5), 173.9(C-2), 163.8 (N=C), 129.0 (C-1"), 130.5 (C-2" and C-6"), 129.0 (C-3" and C-5"), 136.2(C-4").
2c		$C_{16}H_{12}N_3O_2Cl$	185-88	75	1600.5, 1539.9	2904.5, 2826.2, 1375.1	1250.2 (C-O, OCH_3)	137.0 (C-1'), 132.4 (C-2'), 129.5 (C-3'), 129.9 (C-4'), 127.0 (C-5'), 128.5 (C-6'), 169.5 (C-5), 173.8 (C-2), 163.4 (N=C), 123.2 (C-1"), 130.0 (C-2" and C-6"), 114.1 (C-3" and C-5"), 164.5(C-4") and 56.2 (OCH_3),

2d		$C_{15}H_9N_4O_3Cl$	202-205	68	1615.1, 1535.2	2919, 2826, 1382	1537.0, 1345.1 (NO_2),	137.0 (C-1'), 132.4 (C-2'), 129.3 (C-3'), 129.8 (C-4'), 127.1 (C-5'), 128.5 (C-6'), 169.2(C-5), 173.8(C-2), 163.6 (N=C), 126.5 (C-1"), 148.1 (C-2"), 124.0 (C-3"), 131.6(C-4"), 134.6 (C-5") and 130.3(C-6").
2e		$C_{15}H_9N_4O_3Cl$	189-191	65	1620.1, 1539.2	2960.2, 2890.0, 1389.8	1525.7, 1346.9 (NO_2),	136.9 (C-1'), 132.5 (C-2'), 129.4 (C-3'), 130.0 (C-4'), 127.1 (C-5'), 128.5 (C-6'), 168.9(C-5), 174.2(C-2), 163.5 (N=C), 137.4 (C-1"), 129.9 (C-2" and C-6"), 123.7 (C-3" and C-5"), 150.8(C-4").
2f		$C_{13}H_7N_4O_4Cl$	157-159	56	1616	2922.5, 2838.5, 1382.9	1520.9, 1344.2 (NO_2),	136.8 (C-1'), 132.4 (C-2'), 129.4 (C-3'), 130.0 (C-4'), 127.0 (C-5'), 128.4 (C-6'), 168.8(C-5), 173.5 (C-2), 163.5 (N=C), 147.2 (C-2"), 113.2 (C-3"), 113.9 (C-4"), 154.3(C-5").
2g		$C_{19}H_{12}N_3O_2Cl$	143-145	67	1616	2903.2, 2837.5, 1380.4	–	136.7 (C-1'), 132.3 (C-2'), 129.4 (C-3'), 130.0 (C-4'), 127.1 (C-5'), 128.4 (C-6'), 169.4 (C-5), 174.1 (C-2), 163.7 (N=C), 141.1 (C-2"), 110.1 (C-3"), 116.1 (C-4"), 154.3(C-5"), 136.4 (C-1'"), 127.0 (C-2"' and C-6'"), 129.1 (C-3"' and C-5'"), 128.6 (C-4'")
2h		$C_{19}H_{11}N_4O_4Cl$	175-78	58	1616	2921.3, 2837.5, 1382.7	1536.2, 1340.5 (NO_2),	136.8 (C-1'), 132.2 (C-2'), 129.4 (C-3'), 129.9 (C-4'), 127.1 (C-5'), 128.4 (C-6'), 168.8 (C-5), 173.4 (C-2), 163.4 (N=C), 141.5 (C-2"), 110.7 (C-3"), 115.8 (C-4"), 153.5(C-5"), 131.8 (C-1'"), 147.0 (C-2"'), 124.3(C-3'"), 129.3 (C-4"'), 135.2 (C-5'"), 127.8 (C-6'")

*All compounds gave satisfactory CHN analysis.

TABLE-2
in vitro ANTIBACTERIAL AND ANTIFUNGAL
ACTIVITIES OF SCHIFF BASES

Compd.	ALD_{50} (mg/kg i.p.)	Minimum inhibitory concentration (MIC, µg/mL)				
		SA	EC	PA	CA	AF
2a	681	102	108	112	102	120
2b	464	97	100	102	95	100
2c	825	144	180	165	150	190
2d	681	124	110	120	128	145
2e	464	88	70	85	89	78
2f	464	175	160	168	150	136
2g	464	140	140	110	120	126
2h	681	124	128	126	148	150
Ciprofloxacin	-	4	4	6	-	-
Clotrimazole	-	-	-	-	4	8

SA = *S. aureus*; EC = *E. coli*; PA = *P. aeruginosa*; CA = *C. albicans*; AF = *A. fumigatus*.
Data represent mean value for three independent determination; variation among duplicate sample was less than 15 %.

ACKNOWLEDGEMENT

The authors wish to thank Dean, Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan for providing necessary facilities.

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(*Received*: 1 January 2007; *Accepted*: 20 December 2007) AJC-6142